TITLE OF THE INVENTION

DNA MOLECULES ENCODING HG51, A G PROTEIN-COUPLED RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. Serial No. 60/109,717, filed November 24, 1998, which is hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to human DNA molecules encoding HG51, a G protein-coupled receptor (GCPR) having homology to the rhodopsin sub-family of GCPRs, recombinant vectors comprising DNA molecules encoding HG51, recombinant host cells which contain a recombinant vector encoding HG51, the HG51 protein encoded by the DNA molecule, and methods of identifying selective modulators of HG51.

BACKGROUND OF THE INVENTION

G protein-coupled receptors are a large class of membrane receptors that relay information from the exterior to the interior of cells. GPCRs function by interacting with a class of heterotrimeric proteins known as G proteins. Most GPCRs function by a similar mechanism. Upon the binding of an agonist, a GPCR catalyzes the dissociation of guanosine diphosphate (GDP) from the α subunit of G proteins. This allows for the binding of guanosine triphosphate (GTP) to the α subunit, resulting in the disassociation of the α subunit from the β and γ subunits. The freed α subunit then interacts with other cellular components, and in the process passes on the extracellular signal represented by the presence of the agonist. Occasionally, it is the freed β and γ subunits which transduce the agonist signal.

GPCRs possess common structural characteristics. They have seven hydrophobic domains, about 20-30 amino acids long, linked by sequences of hydrophilic amino acids of varied length. These seven hydrophobic domains intercalate into the plasma membrane, giving rise to a protein with seven transmembrane domains, an extracellular amino terminus, and an intracellular carboxy terminus (Strader et al., 1994, *Ann. Rev. Biochem.* 63:101-132; Schertler et al., 1993, *Nature* 362:770-7721; Dohlman et al., 1991, *Ann. Rev. Biochem.* 60:653-688).

GPCRs are expressed in a wide variety of tissue types and respond to a wide range of ligands, *e.g.*, protein hormones, biogenic amines, peptides, lipid derived messengers, etc. Given their wide range of expression and ligands, it is not surprising that GPCRs are involved in many pathological states. This has led to great interest in developing modulators of GPCR activity that can be used pharmacologically. For example, Table 1 of Stadel et al. (1997, *Trends Pharmacol. Sci.* 18:430-437), lists 37 different marketed drugs that act upon GPCRs. Accordingly, there is a great need to understand GPCR function and to develop agents that can be used to modulate GPCR activity.

Rhodopsin receptors possess the seven transmembrane helices which characterize other GPCRs. Rhodopsin receptors comprise a chromophore-binding pocket which is covalently linked by a protonated Schiff base to a Lys residue in TM 7. For a review of rhodopsin receptors, see Sakmar, 1998, *Progress in Nucleic Acid Research and Molecular Biology* 59: 1-33.

It would be advantageous to identify novel members of the rhodopsin subtype of GPCRs which are expressed in a wide variety of tissue and are involved in important metabolic functions. The present invention addresses and meets these needs by disclosing an isolated nucleic acid fragment which expresses a form of human HG51, recombinant vectors which house this nucleic acid fragment, recombinant host cells which expresses human HG51 and/or a biologically active equivalent, and pharmacological properties of this human HG51 protein.

SUMMARY OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid molecule (polynucleotide) which encodes a novel G protein-coupled receptor, HG51. The nucleic acid molecules of the present invention are substantially free from other nucleic acids.

The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel G protein-coupled receptor, HG51, this DNA molecule comprising the nucleotide sequence disclosed herein as SEQ ID NO:1.

The present invention also relates to biologically active fragments or mutants of SEQ ID NO:1 which encodes mRNA expressing a novel G protein-coupled receptor, HG51. Any such biologically active fragment and/or mutant will

encode either a protein or protein fragment which at least substantially mimics the pharmacological properties of a wild-type, HG51 protein, including but not limited to the HG51 receptor protein as set forth in SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for agonists and/or antagonists for HG51 function.

A preferred aspect of this portion of the present invention is disclosed in Figure 1, a human cDNA molecule encoding a novel HG51 (SEQ ID NO:1).

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification.

The present invention also relates to subcellular membrane fractions of the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) which contain the proteins encoded by the nucleic acids of the present invention. These subcellular membrane fractions will comprise either wild-type or mutant forms of human HG51 proteins at levels substantially above endogenous levels and hence will be useful in various assays described throughout this specification.

The present invention also relates to a substantially purified form of the human HG51 receptor protein, which comprises the amino acid sequence disclosed in Figure 2 and set forth as SEQ ID NO:2.

The present invention also relates to biologically active fragments and/or mutants of the G protein-coupled receptor, HG51, comprising the amino acid sequence as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein

fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators, including but not limited to agonists and/or antagonists for HG51 function.

A preferred aspect of the present invention is disclosed in Figure 2 and is set forth as SEQ ID NO:2, the amino acid sequence of the novel human G protein-coupled receptor, HG51. HG51 is a novel member of the rhodopsin GPCR family. It is most homologous to the light receptors (opsin) in the eye. Opsins use retinol as the natural ligand. HG51 contains a lysine residue that is critical to form the Schiff base in opsin, suggesting that the ligand of HG51 may be a fatty-acid-like molecule. Since HG51 is expressed in a wide variety of tissue, it should have important functions in metabolism. Potential therapeutic targets of HG51 include but are not limited to obesity and type II diabetes, in view of the possible receptor function involving fatty acid derivatives which are important in obesity/diabetes. In addition, *in situ* data of HG51 suggests that this gene is highly expressed in certain cells of the colon. Therefore, other potential therapeutic targets of HG51 include but are not limited to various GI diseases such as inflammatory bowel disease, constipation and diarrhea.

The present invention also relates to polyclonal and monoclonal antibodies raised in response to either the human form of HG51, or a biologically active fragment thereof.

The present invention also relates to isolated nucleic acid molecules which are fusion constructions expressing fusion proteins useful in assays to identify compounds which modulate wild-type vertebrate HG51.

Therefore, the present invention relates to methods of expressing the human HG51 receptor protein and biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these receptor proteins, and compounds identified through these assays which act as agonists or antagonists of HG51 activity.

The present invention also relates to assays to screen or select for various modulators of HG51 activity, methods of expressing the HG51 protein and biological equivalents disclosed herein, recombinant host cells which comprise DNA constructs which express these receptor proteins, and compounds identified through these assays which act as agonists or antagonists of HG51 activity.

It is an object of the present invention to provide an isolated nucleic acid molecule which encodes a novel form of human HG51, or human HG51

fragments, mutants or derivatives of SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators for vertebrate HG51 function.

It is a further object of the present invention to provide the human HG51 proteins or protein fragments encoded by the nucleic acid molecules referred to in the preceding paragraph.

It is a further object of the present invention to provide recombinant vectors and recombinant host cells which comprise a nucleic acid sequence encoding human HG51 or a biological equivalent thereof.

It is an object of the present invention to provide a substantially purified form of the human HG51 protein, as set forth in SEQ ID NO:2.

It is an object of the present invention to provide for biologically active fragments and/or mutants of the human HG51 protein, such as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic and/or prophylactic use.

It is also an object of the present invention to provide for HG51-based in-frame fusion constructions, methods of expressing these fusion constructs, biological equivalents disclosed herein, related assays, recombinant cells expressing these constructs, and agonistic and/or antagonistic compounds identified through the use of the nucleic acid encoding vertebrate, mammalian and/or human HG51 protein as well as the expressed protein.

It is also an object of the present invention to provide for HG51-based assays to select for modulators of this receptor protein. These assays are preferably cell based assays whereby a DNA molecule encoding HG51 is transfected or transformed into a host cell, this recombinant host cell is allowed to grow for a time sufficient to express HG51 prior to use in various assays described herein.

It is a further object to provide for membrane preparations from host cells transfected or transformed with a DNA molecule encoding HG51 for use in assays to select for modulators of HG51 activity.

Therefore, it is an object of the present invention to use HG51, cells transfected with an expression vector which directs the expression of HG51 or membrane preparations containing HG51 or a biological equivalent to screen for modulators, preferably selective modulators, of HG51 activity. Any such compound may be useful in diagnostic, therapeutic and/or prophylactic indications for such disease states including but not limited to obesity, type II diabetes and various GI diseases including but not limited to inflammatory bowel disease, constipation and diarrhea.

As used herein, "substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, an HG51 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG51 proteins. Whether a given HG51 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

As used herein, "substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, an HG51 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG51 nucleic acids. Whether a given HG51 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

As used interchangeably herein, "functional equivalent" or "biologically active equivalent" means a receptor which does not have exactly the same amino acid sequence as naturally occurring HG51, due to alternative splicing, deletions, mutations, substitutions, or additions, but retains substantially the same biological activity as HG51. Such functional equivalents will have significant amino acid sequence identity with naturally occurring HG51 and genes and cDNA encoding

such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring HG51. For the purposes of this invention, naturally occurring HG51 has the amino acid sequence shown as SEQ ID NO:2 and is encoded by SEQ ID NO:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ ID NO:1.

A polypeptide has "substantially the same biological activity" as HG51 if that polypeptide has a K_d for a ligand that is no more than 5-fold greater than the K_d of HG51 having SEQ ID NO:2 for the same ligand.

As used herein, "a conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid).

As used interchangeably herein, "isolated HG51 protein" or "purified HG51 protein" refers to HG51 protein that has been isolated from a natural source. Use of the term "isolated" or "purified" indicates that HG51 protein has been removed from its normal cellular environment. Thus, an isolated HG51 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated HG51 protein is the only protein present, but instead means that an isolated HG51 protein is substantially free of other proteins and non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the HG51 protein *in vivo*. Thus, an HG51 protein that is expressed in a prokaryotic or eukaryotic cell which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated HG51 protein."

As used herein, "GPCR" refers to --G protein-coupled receptor--.

As used herein, the term "mammalian host" will refer to any mammal, including a human being.

BRIEF DECRIPTION OF THE FIGURES

Figure 1 shows the nucleotide sequence which encodes human HG51, as set forth in SEQ ID NO:1.

Figure 2 shows the amino acid sequence of human HG51, as set forth in SEQ ID NO:2.

Figures 3A, 3B, and 3C show the translation of the HG51 open reading frame. The nucleotide sequence shown is as set forth in SEQ ID NO:1. The amino acid sequence shown is as set forth in SEQ ID NO:2.

Figures 4A, 4B, 4C, 4D, and 4E show multi-tissue Northern analysis of mRNA which encodes human HG51

Figure 5 shows an alignment of a portion of the amino acid sequence of HG51 (as contained within SEQ ID NO:2) with the amino acid sequence of the human rhodopsin receptor (SEQ ID NO:15).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes a novel human GPCR, HG51, which shows homology to the human rhodopsin receptor. The nucleic acid molecules of the present invention are substantially free from other nucleic acids. For most cloning purposes, DNA is a preferred nucleic acid.

The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel human HG51 GPCR, this DNA molecule comprising the nucleotide sequence disclosed herein as SEQ ID NO:1, shown herein as follows:

GGGGCCACGG GGGGTGCGCC GGCGCGCGGT AGCGCGGGCC CCTCAGTGCA CAATGGCCAG
AGCAGGCGGC GGAGCCCCAG CCCCACCCAG TGCGGAGCGC GCCGCGAGCC CCGCCGCAAG
CTGAGCGCCT CCGCCCGCCA GGCGCGCCGG CGCCGGGCCA TGTACTCGGG GAACCGCAGC
GGCGGCCACG GCTACTGGGA CGGCGGCGGG GCCGCGGGCG CTGAGGGGCC GGCGCCGGCG
GGGACACTGA GCCCCGCGCC CCTCTTCAGC CCCGGCACCT ACGAGCGCCT GGCGCTGCTG
CTGGGCTCCA TTGGGCTGCT GGGCGTCGGC AACAACCTGC TGGTGCTCGT CCTCTACTAC
AAGTTCCAGC GGCTCCGCAC TCCCACTCAC CTCCTCCTGG TCAACATCAG CCTCAGCGAC
CTGCTGGTGT CCCTCTTCGG GGTCACCTTT ACCTTCGTGT CCTGCCTGAG GAACGGCTGG
GTGTGGGACA CCGTGGGCTG CGTGTGGGAC GGGTTTAGCG GCAGCCTCTT CGGGATTGTT
TCCATTGCCA CCCTAACCGT GCTGGCCTAT GAACGTTACA TTCGCGTGGT CCATGCCAGA
GTGATCAATT TTTCCTGGGC CTGGAGGGCC ATTACCTACA TCTGGCTCTA CTCACTGGCG
TGGGCAGGAG CACCTCTCCT GGGATGGAAC AGGTACATCC TGGACGTACA CGGACTAGGC
TGCACTGTGG ACTGGAAATC CAAGGATGCC AACGATTCCT CCTTTGTGCT TTTCTTATTT

CTTGGCTGCC TGGTGGTGCC CCTGGGTGTC ATAGCCCATT GCTATGGCCA TATTCTATAT
TCCATTCGAA TGCTTCGTTG TGTGGAAGAT CTTCAGACAA TTCAAGTGAT CAAGATTTTA
AAATATGAAA AGAAACTGGC CAAAATGTGC TTTTTAATGA TATTCACCTT CCTGGTCTGT
TGGATGCCTT ATATCGTGAT CTGCTTCTTG GTGGTTAATG GTCATGGTCA CCTGGTCACT
CCAACAATAT CTATTGTTTC GTACCTCTTT GCTAAATCGA ACACTGTATA CAATCCAGTG
ATTTATGTCT TCATGATCAG AAAGTTTCGA AGATCCCTTT TGCAGCTTCT GTGCCTCCGA
CTGCTGAGGT GCCAGAGGCC TGCTAAAGAC CTACCAGCAG CTGGAAGTGA AATGCAGATC
AGACCCATTG TGATGTCACA GAAAGATGGG GACAGGCCAA AGAAAAAAGT GACTTTCAAC
TCTTCTTCCA TCATTTTTAT CATCACCAGT GATGAATCAC TGTCAGTTGA CGACAGCGAC
AAAACCAATG GGTCCAAAGT TGATGTAATC CAAGTTCGTC CTTTGTAGGA ATGAAGAATG
GCAACGAAAG ATGGGGCCTT AAATTGGATG CCACTTTTGG ACTTTCATCA TAAGAAGTGT
CTGGAATACC CGTTCTATGT AATATCAACA GAACCTTGTG GTCCAGCAGG AAATCCGAAT
TGCCCATATG CTCTTGGGCC TCAGGAAGAG GTTGAAC (SEQ ID NO:1)

The above-exemplified isolated DNA molecule, shown in Figure 1 and set forth as SEQ ID NO:1, contains 1537 nucleotides. This DNA molecule contains an open reading frame from nucleotide 160 to nucleotide 1365, with a "TAG" termination codon from nucleotides 1366-1368. This open reading frame encodes a human HG51 GPCR, which shares homology to human rhodopsin. The HG51 protein contains an open reading frame of 402 amino acids in length, as shown in Figure 2 and as set forth in SEQ ID NO:2. One partial cDNA sequence (EST) (Genbank accession number aa745052) was found by searching the EST database using protein sequences of G protein-coupled receptors. DNA sequence information of this EST was then used to isolate cDNA fragments containing the original ESTs. DNA sequences of these fragments were determined and analyzed, resulting in the identification of the full-length coding sequence of the gene designated HG51. Northern analysis of multi-tissue mRNA blots showed that HG51 was weakly expressed in many tissues as transcripts of ~8.0 - 9.0 kb and ~2.0 - 2.5 kb. Expression of HG51 in HEK293 cells led to the increase in intracellular cAMP level, indicating that HG51 is coupled to the Gs protein.

The present invention also relates to biologically active fragments or mutants of SEQ ID NO:1 which encodes mRNA expressing HG51. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the pharmacological properties of human HG51 protein, including but not limited to the human HG51 receptor protein as set

forth in SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for agonists and/or antagonists for HG51 function.

A preferred aspect of this portion of the present invention is disclosed in Figure 1, a cDNA molecule encoding human HG51 (SEQ ID NO:1).

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the HG51 protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ ID NO: 1, but still encodes the same HG51 protein as SEQ ID NO: 1. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus leading to higher levels of expression of HG51 protein in the host. In other words, this redundancy in the various codons which code for specific amino acids is within the scope of the present invention. Therefore, this invention is also directed to those DNA sequences which encode RNA comprising alternative codons which code for the eventual translation of the identical amino acid, as shown below:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
C=Cys=Cysteine: codons UGC, UGU
D=Asp=Aspartic acid: codons GAC, GAU
E=Glu=Glutamic acid: codons GAA, GAG
F=Phe=Phenylalanine: codons UUC, UUU
G=Gly=Glycine: codons GGA, GGC, GGG, GGU
H=His =Histidine: codons CAC, CAU

I=Ile =Isoleucine: codons AUA, AUC, AUU
K=Lys=Lysine: codons AAA, AAG
L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
M=Met=Methionine: codon AUG
N=Asp=Asparagine: codons AAC, AAU
P=Pro=Proline: codons CCA, CCC, CCG, CCU
Q=Gln=Glutamine: codons CAA, CAG
R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU
S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU
T=Thr=Threonine: codons ACA, ACC, ACG, ACU
V=Val=Valine: codons GUA, GUC, GUG, GUU
W=Trp=Tryptophan: codon UGG
Y=Tyr=Tyrosine: codons UAC, UAU

Therefore, the present invention discloses codon redundancy which may result in differing DNA molecules expressing an identical protein. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations either in the DNA sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide having properties that are different than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but are not limited to changes in the affinity of an enzyme for a substrate or a receptor for a ligand.

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification. The nucleic acid molecules of the present invention encoding HG51, in whole or in part, can be linked with other DNA molecules, i.e., DNA molecules to which the human HG51 are not naturally linked, to form "recombinant DNA molecules" containing the

receptor. The novel DNA sequences of the present invention can be inserted into vectors which comprise nucleic acids encoding human HG51 or a functional equivalent.

These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a HG51 receptor protein. It is well within the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

Included in the present invention are DNA sequences that hybridize to SEQ ID NO:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hours at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 minutes before autoradiography. Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The present invention also relates to a substantially purified form of the human HG51 receptor protein, which comprises the amino acid sequence disclosed in Figure 2 and as set forth in SEQ ID NO:2.

The present invention also relates to biologically active fragments and/or mutants of the human HG51 receptor protein comprising the amino acid sequence as set forth in SEQ ID NO:2, including but not necessarily limited to amino

acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for agonists and/or antagonists of HG51 function.

A preferred aspect of the present invention is disclosed in Figure 2 and is set forth as SEQ ID NO:2 in three letter code, and as herein set forth as follows in one letter code:

MYSGNRSGGH GYWDGGGAAG AEGPAPAGTL SPAPLFSPGT YERLALLLGS IGLLGVGNNL
LVLVLYYKFQ RLRTPTHLLL VNISLSDLLV SLFGVTFTFV SCLRNGWVWD TVGCVWDGFS
GSLFGIVSIA TLTVLAYERY IRVVHARVIN FSWAWRAITY IWLYSLAWAG APLLGWNRYI
LDVHGLGCTV DWKSKDANDS SFVLFLFLGC LVVPLGVIAH CYGHILYSIR MLRCVEDLQT
IQVIKILKYE KKLAKMCFLM IFTFLVCWMP YIVICFLVVN GHGHLVTPTI SIVSYLFAKS
NTVYNPVIYV FMIRKFRRSL LQLLCLRLLR CQRPAKDLPA AGSEMQIRPI VMSQKDGDRP
KKKVTFNSSS IIFIITSDES LSVDDSDKTN GSKVDVIQVR PL (SEQ ID NO:2), which comprises the amino acid sequence of wild type human HG51 receptor protein.

As with many receptor proteins, it is possible to modify many of the amino acids of HG51, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus this invention includes modified HG51 polypeptides which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as HG51. Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ ID NO:2 wherein the polypeptides still retain substantially the same biological activity as HG51. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ ID NO:2 wherein the polypeptides still retain substantially the same biological activity as HG51. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of HG51.

When deciding which amino acid residues of HG51 may be substituted to produce polypeptides that are functional equivalents of HG51, one skilled in the art would be guided by a comparison of the amino acid sequence of HG51 with the amino acid sequences of related proteins, *e.g.*, the human rhodopsin receptor as

shown in Figure 5 of the present specification. Such a comparison would allow one skilled in the art to minimize the number of amino acid substitutions made in regions that are highly conserved between HG51 and the related protein. Accordingly, the present invention includes embodiments where the substitutions are conservative and do not occur in positions where HG51 and the human rhodopsin receptor share the same amino acid (again, see Figure 5).

One skilled in the art would also recognize that polypeptides that are functional equivalents of HG51 and have changes from the HG51 amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, (i.e., minimizing the differences in amino acid sequence between HG51 and related proteins). Small deletions or insertions are generally in the range of about 1 to 5 amino acids. The effect of such small deletions or insertions on the biological activity of the modified HG51 polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding HG51 and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression.

The present invention also includes C-terminal truncated forms of HG51, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

The present invention also includes chimeric HG51 proteins. Chimeric HG51 proteins consist of a contiguous polypeptide sequence of HG51 fused in frame to a polypeptide sequence of a non-HG51 protein. For example, the N-terminal domain and seven transmembrane spanning domains of HG51 fused at the C-terminus in frame to a G protein would be a chimeric HG51 protein.

The present invention also includes HG51 proteins that are in the form of multimeric structures, e.g., dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, *J. Biol. Chem.* 271, 16384-16392; Ng *et al.*, 1996, *Biochem. Biophys. Res. Comm.* 227, 200-204; Romano *et al.*, 1996, *J. Biol. Chem.* 271, 28612-28616).

The present invention also relates to subcellular membrane fractions from the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) which contain the nucleic acid molecules of

the present invention. These recombinant host cells express HG51 or a functional equivalent, which becomes associated with the appropriate membrane (such as the cell membrane) in a biologically active fashion. These subcellular membrane fractions will comprise either wild-type or mutant forms of human HG51 receptor proteins at levels substantially above endogenous levels and hence will be useful in various assays described throughout this specification.

The present invention also relates to polyclonal and monoclonal antibodies raised in response to either the human form of HG51, or a biologically active equivalent thereof.

The present invention also relates to isolated nucleic acid molecules which are fusion constructions expressing fusion proteins useful in assays to identify compounds which modulate wild-type vertebrate HG51 activity. A preferred aspect of this portion of the invention includes, but is not limited to, glutathione S-transferase (GST)-HG51 fusion constructs which include, but are not limited to, either the intracellular domain of human HG51 as an in-frame fusion at the carboxy terminus of the GST gene or the extracellular and transmembrane ligand binding domain of HG51 fused to an GST or immunoglobulin gene by methods known to one of ordinary skill in the art. Recombinant GST-HG51 fusion proteins may be expressed in various expression systems, including *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen).

Based on its predicted amino acid sequence, the HG51 protein represents a novel G-protein coupled receptor (GPCR) since the HG51 protein contains many of the characteristic features of G-protein coupled receptors (GPCRs), including but not necessarily limited to (a) seven transmembrane domains; (b) homology with members of the rhodopsin family of GPCRs; and, (c) signature motifs of GPCRs in the rhodopsin family, such as a conserved NPXXY motif in transmembrane domain 7 (amino acid residue 305 to 309).

As with many receptor proteins, it is possible to modify many of the amino acids, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus this invention includes modified HG51 polypeptides which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as HG51. It is generally accepted that single amino acid substitutions do not usually

alter the biological activity of a protein (see, e.g., *Molecular Biology of the Gene*, Watson et al., 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, *Science* 244:1081-1085). Accordingly, the present invention includes isolated nucleic acid molecules and expressed HG51 proteins wherein one amino acid substitution is generated and which this protein retains substantially the same biological activity as wild-type HG51. The present invention also includes isolated nucleic acid molecules and expressed HG51 proteins wherein two or more amino acid substitution is generated wherein this protein retains substantially the same biological activity as wild-type HG51. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of HG51.

Any of a variety of procedures may be used to clone human HG51. These methods include, but are not limited to, (1) a RACE PCR cloning technique (Frohman, et al., 1988, *Proc. Natl. Acad. Sci. USA* 85: 8998-9002). 5′ and/or 3′ RACE may be performed to generate a full-length cDNA sequence. This strategy involves using gene-specific oligonucleotide primers for PCR amplification of human HG51 cDNA. These gene-specific primers are designed through identification of an expressed sequence tag (EST) nucleotide sequence which has been identified by searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the human HG51 cDNA following the construction of a human HG51-containing cDNA library in an appropriate expression vector system; (3) screening a human HG51-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from the amino acid sequence of the human HG51 protein; (4) screening a human HG51-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the human HG51 protein. This partial cDNA is obtained by the specific PCR amplification of human HG51 DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other kinases which are related to the human HG51 protein; (5) screening a human HG51-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA or oligonucleotide with homology to a mammalian HG51 protein. This strategy may also involve using gene-specific oligonucleotide

primers for PCR amplification of human HG51 cDNA identified as an EST as described above; or (6) designing 5′ and 3′ gene specific oligonucleotides using SEQ ID NO: 1 as a template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding human HG51.

It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types-or species types, may be useful for isolating a human HG51-encoding DNA or a human HG51 homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other rhesus cells.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have HG51 activity. The selection of cells or cell lines for use in preparing a cDNA library to isolate a cDNA encoding human HG51 may be done by first measuring cell-associated HG51 activity using any known assay available for such a purpose.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Complementary DNA libraries may also be obtained from numerous commercial sources, including but not limited to Clontech Laboratories, Inc. and Stratagene.

It is also readily apparent to those skilled in the art that DNA encoding human HG51 may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in Sambrook, et al., *supra*. Genomic clones containing the HG51 gene can be obtained from commercially available human PAC or BAC libraries, *e.g.*, from Research Genetics, Huntsville, AL. Alternatively, one may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the HG51 can be isolated, using probes based upon the HG51 nucleotide

sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou et al.,1994, *Nature Genet.* 6:84-89).

In order to clone the human HG51 gene by one of the preferred methods, it may be necessary to obtain at least a portion of the amino acid sequence or DNA sequence of human HG51 or a homologous protein. To accomplish this, the HG51 protein or a homologous protein may be purified and partial amino acid sequence determined by automated sequenators. It is not necessary to determine the entire amino acid sequence, but the linear sequence of two regions of 6 to 8 amino acids can be determined for the PCR amplification of a partial human HG51 DNA fragment. Once suitable amino acid sequences have been identified, the DNA sequences capable of encoding them are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the human HG51 sequence but others in the set will be capable of hybridizing to human HG51 DNA even in the presence of DNA oligonucleotides with mismatches. The mismatched DNA oligonucleotides may still sufficiently hybridize to the human HG51 DNA to permit identification and isolation of human HG51 encoding DNA. Alternatively, the nucleotide sequence of a region of an expressed sequence may be identified by searching one or more available genomic databases. Gene-specific primers may be used to perform PCR amplification of a cDNA of interest from either a cDNA library or a population of cDNAs. As noted above, the appropriate nucleotide sequence for use in a PCR-based method may be obtained from SEQ ID NO: 1, either for the purpose of isolating overlapping 5′ and 3′ RACE products for generation of a full-length sequence coding for human HG51, or to isolate a portion of the nucleotide sequence coding for human HG51 for use as a probe to screen one or more cDNA- or genomic-based libraries to isolate a full-length sequence encoding human HG51 or human HG51-like proteins.

This invention also includes vectors containing a HG51 gene, host cells containing the vectors, and methods of making substantially pure HG51 protein comprising the steps of introducing the HG51 gene into a host cell, and cultivating the host cell under appropriate conditions such that HG51 is produced. The HG51 so produced may be harvested from the host cells in conventional ways. Therefore, the present invention also relates to methods of expressing the human HG51 protein and

biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these receptor proteins, and compounds identified through these assays which act as agonists or antagonists of HG51 activity.

The cloned human HG51 cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector (such as pcDNA3.neo, pcDNA3.1, pCR2.1, pBlueBacHis2 or pLITMUS28) containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant human HG51. Techniques for such manipulations can be found described in Sambrook, et al., *supra*, are discussed in the Example sections and are well known and easily available to the artisan of ordinary skill in the art. Therefore, another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding the HG51. An expression vector containing DNA encoding a human HG51-like protein may be used for expression of human HG51 in a recombinant host cell. Such recombinant host cells can be cultured under suitable conditions to produce HG51 or a biologically equivalent form. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines.

For instance, one insect expression system utilizes *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) in tandem with a baculovirus expression vector (pAcG2T, Pharmingen). Also, mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M (TK^-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), Saos-2 (ATCC HTB-85), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171) and CPAE (ATCC CCL 209).

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary (CHO) cells are particularly suitable for expression of the HG51 protein because these cells express a large number of G-proteins. Thus, it is likely that at least one of these

G-proteins will be able to functionally couple the signal generated by interaction of HG51 and its ligands, thus transmitting this signal to downstream effectors, eventually resulting in a measurable change in some assayable component, *e.g.*, cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or intracellular Ca^{++} levels.

Other cells that are particularly suitable for expression of the HG51 protein are immortalized melanophore pigment cells from *Xenopus laevis*. Such melanophore pigment cells can be used for functional assays using recombinant expression of HG51 in a manner similar to the use of such melanophore pigment cells for the functional assay of other recombinant GPCRs (Graminski et al., 1993, *J. Biol. Chem.* 268:5957-5964; Lerner, 1994, *Trends Neurosci.* 17:142-146; Potenza & Lerner, 1992, *Pigment Cell Res.* 5: 372-378).

A variety of mammalian expression vectors may be used to express recombinant human HG51 in mammalian cells. Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned DNA and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic DNA in a variety of hosts such as bacteria, blue green algae, plant cells, insect cells and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency.

Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available mammalian expression vectors which may be suitable for recombinant human HG51 expression, include but are not limited to, pIRES-hyg (Clontech), pIRES-puro (Clontech), pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pCI-neo (Promega), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Bioloabs), pcDNAI, pcDNAIamp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12)

(ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lZD35 (ATCC 37565).

Also, a variety of bacterial expression vectors may be used to express recombinant human HG51 in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant human HG51 expression include, but are not limited to pCR2.1 (Invitrogen), pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia).

In addition, a variety of fungal cell expression vectors may be used to express recombinant human HG51 in fungal cells. Commercially available fungal cell expression vectors which may be suitable for recombinant human HG51 expression include but are not limited to pYES2 (Invitrogen) and *Pichia* expression vector (Invitrogen).

Also, a variety of insect cell expression vectors may be used to express recombinant receptor in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of human HG51 include but are not limited to pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (Pharmingen).

The assays described herein can be carried out with cells that have been transiently or stably transfected or transformed with an expression vector which directs expression of HG51. The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation. Transformation is meant to encompass a genetic change to the target cell resulting from an incorporation of DNA. Transfection is meant to include any method known in the art for introducing HG51 into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, electroporation, as well as infection with, for example, a viral vector such as a recombinant retroviral vector containing the nucleotide sequence which encodes HG51, and combinations thereof. The expression vector-containing cells are individually analyzed to determine whether they produce human HG51 protein. Identification of human HG51 expressing cells may be done by several means, including but not limited to immunological reactivity with anti-human HG51 antibodies, labeled ligand binding and the presence of host cell-associated human HG51 activity.

Expression of human HG51 DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

To determine the human HG51 cDNA sequence(s) that yields optimal levels of human HG51, cDNA molecules including but not limited to the following can be constructed: a cDNA fragment containing the full-length open reading frame for human HG51 as well as various constructs containing portions of the cDNA encoding only specific domains of the protein or rearranged domains of the protein. All constructs can be designed to contain none, all or portions of the 5′ and/or 3′ untranslated region of a human HG51 cDNA. The expression levels and activity of human HG51 can be determined following the introduction, both singly and in combination, of these constructs into appropriate host cells. Following determination of the human HG51 cDNA cassette yielding optimal expression in transient assays, this HG51 cDNA construct is transferred to a variety of expression vectors (including recombinant viruses), including but not limited to those for mammalian cells, plant cells, insect cells, oocytes, bacteria, and yeast cells.

Following expression of human HG51 in a host cell, HG51 protein may be recovered to provide HG51 protein in active form. Several HG51 protein purification procedures are available and suitable for use. Recombinant HG51 protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant HG51 protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length HG51 protein, or polypeptide fragments of HG51 protein.

The present invention is also directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a human HG51 protein.

The present invention relates to assays by which HG51 agonists and antagonists may be identified. Methods for identifying agonists and antagonists of

other receptors are well known in the art and can be adapted to identify agonists and antagonists of HG51. For example, Cascieri et al. (1992, *Molec. Pharmacol.* 41:1096-1099) describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor.

Where binding of the substance such as an agonist or antagonist to HG51 is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

Therefore, the present invention relates to methods of expressing HG51 in recombinant systems and of identifying agonists and antagonists of HG51. The novel HG51 receptor protein of the present invention is suitable for use in an assay procedure for the identification of compounds which modulate the receptor activity. Modulating receptor activity, as described herein includes the inhibition or activation of the receptor and also includes directly or indirectly affecting the normal regulation of the receptor activity. Compounds which modulate the receptor activity include agonists, antagonists and compounds which directly or indirectly affect regulation of the receptor activity. When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target receptor, it is necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find compounds that are potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus target") and produce the desired pharmacological effect through receptor A, it is also

necessary to determine that the compounds do not interact with receptors B, C, D, etc. (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, *Bio/Technology* 10:973-980, @ 980). Human HG51 proteins and the DNA molecules encoding this receptor protein have the additional utility in that they can be used as "minus targets" in screens designed to identify compounds that specifically interact with other G-protein coupled receptors.

The specificity of binding of compounds having affinity for HG51 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to HG51 or that inhibit the binding of a known, radiolabeled ligand of HG51 to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for HG51. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of HG51 and may be peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding human HG51, or by acting as an agonist or antagonist of HG51 receptor protein. These compounds that modulate the expression of DNA or RNA encoding human HG51 or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample. Kits containing human HG51, antibodies to human HG51, or modified human HG51 may be prepared by known methods for such uses.

To this end, the present invention relates in part to methods of identifying a substance which modulates HG51 receptor activity, which involves:

(a) combining a test substance in the presence and absence of a HG51 receptor protein wherein said HG51 receptor protein comprises the amino acid sequence as set forth in SEQ ID NO:2; and,

(b) measuring and comparing the effect of the test substance in the presence and absence of the HG51 receptor protein.

In addition, several specific embodiments are disclosed herein to show the diverse type of screening or selection assay which the skilled artisan may utilize in tandem with an expression vector directing the expression of the HG51 receptor protein. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of HG51. Therefore, these embodiments are presented as examples and not as limitations. To this end, the present invention includes assays by which HG51 modulators (such as agonists and antagonists) may be identified. Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of HG51 that comprises:

(a) transfecting or transforming cells with an expression vector that directs expression of HG51 in the cells, resulting in test cells;

(b) allowing the test cells to grow for a time sufficient to allow HG51 to be expressed;

(c) exposing the cells to a labeled ligand of HG51 in the presence and in the absence of the substance; and,

(d) measuring the binding of the labeled ligand to HG51; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG51.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C. The test cells may be harvested and resuspended in the presence of the substance and the labeled ligand. In a modification of the above-described method, step (c) is modified in that the cells are not harvested and resuspended but rather the radioactively labeled known agonist and the substance are contacted with the cells while the cells are attached to a substratum, e.g., tissue culture plates.

The present invention also includes a method for determining whether a substance is capable of binding to HG51, i.e., whether the substance is a potential agonist or an antagonist of HG51, where the method comprises:

(a) transfecting or transforming cells with an expression vector that directs the expression of HG51 in the cells, resulting in test cells;

(b) exposing the test cells to the substance; and,

(c) measuring the amount of binding of the substance to HG51;

(d) comparing the amount of binding of the substance to HG51 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG51;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG51. Determining whether the substance is actually an agonist or antagonist can then be accomplished by the use of functional assays such as, e.g., the assay involving the use of promiscuous G-proteins described below.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C. The test cells are harvested and resuspended in the presence of the substance.

Chen et al. (1995, *Analytical Biochemistry* 226: 349-354) describe a colorometric assay which utilizes a recombinant cell transfected with an expression vector encoding a G-protein coupled receptor with a second expression vector containing a promoter with a cAMP responsive element fused to the LacZ gene. Activity of the overexpressed G-protein coupled receptor is measured as the expression and OD measurement of ß-Gal. Therefore, another aspect of this portion of the invention includes a non-radioactive method for determining whether a substance is a potential agonist or antagonist of HG51 that comprises:

(a) stably transfecting or transforming cells with an expression vector encoding HG51;

(b) transiently or stably transfecting the recombinant host cell line of step (a) with an expression vector which comprises a cAMP-inducible promoter fused to a colorometric gene such a LacZ;

(c) allowing the transfected cells to grow for a time sufficient to allow HG51 to be expressed;

(d) harvesting the transfected cells and resuspending the cells in the presence of a known agonist of HG51 and/or in both the presence and absence of the test compound; and,

(e) measuring the binding of the known agonist and test compound to overexpressed HG51 by a colorometric assay which measures expression off the cAMP-inducible promoter and comparing expression levels in the presence of the known agonist as well as in the presence and absence of the unknown substance so as to determine whether the unknown substance acts as either a potential agonist or antagonist of HG51.

Additional methods of identifying agonists or antagonists include but are by no means limited to the following:

I. (a) transfecting or transforming cells with a first expression vector which directs expression of HG51 and a second expression vector which directs the expression of a promiscuous G-protein, resulting in test cells;

(b) exposing the test cells to a substance that is a suspected agonist of HG51; and,

(c) measuring the level of inositol phosphates in the cells; where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of HG51.

II. (a) transfecting or transforming cells with a first expression vector which directs expression of HG51 and a second expression vector which directs the expression of a promiscuous G-protein, resulting in test cells;

(b) exposing the test cells to a substance that is an agonist of HG51;

(c) subsequently or concurrently to step (b), exposing the test cells to a substance that is a suspected antagonist of HG51; and,

(d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of HG51.

III. The method of II wherein the first and second expression vectors of step (a) are replaced with a single expression vector which expresses a chimeric HG51 protein fused at its C-terminus to a promiscuous G-protein.

The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells

and those membranes can be exposed to the substance. Such a modification utilizing membranes rather than cells is well known in the art and is described in, e.g., Hess et al., 1992, *Biochem. Biophys. Res. Comm.* 184:260-268. Accordingly, another embodiment of the present invention includes a method for determining whether a substance binds and/or is a potential agonist or antagonist of HG51 wherein membrane preparations from the test cells are utilized in place of the test cells. Such methods comprise the following and may utilized the physiological conditions as noted above:

(a) transfecting or transforming cells with an expression vector that directs the expression of HG51 in the cells, resulting in test cells;

(b) preparing membranes containing HG51 from the test cells and exposing the membranes to a ligand of HG51 under conditions such that the ligand binds to the HG51 in the membranes;

(c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;

(d) measuring the amount of binding of the ligand to the HG51 in the membranes in the presence and the absence of the substance; and,

(e) comparing the amount of binding of the ligand to HG51 in the membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG51 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG51.

The present invention also relates to a method for determining whether a substance is capable of binding to HG51 comprising:

(a) transfecting or transforming cells with an expression vector that directs the expression of HG51 in the cells, resulting in test cells;

(b) preparing membranes containing HG51 from the test cells and exposing the membranes from the test cells to the substance;

(c) measuring the amount of binding of the substance to the HG51 in the membranes from the test cells; and,

(d) comparing the amount of binding of the substance to HG51 in the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG51, where if the amount of binding of the substance to HG51 in the membranes from the test cells is

greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG51.

Polyclonal or monoclonal antibodies may be raised against human HG51 or a synthetic peptide (usually from about 9 to about 25 amino acids in length) from a portion of human HG51 as disclosed in SEQ ID NO:2. Monospecific antibodies to human HG51 are purified from mammalian antisera containing antibodies reactive against human HG51 or are prepared as monoclonal antibodies reactive with human HG51 using the technique of Kohler and Milstein (1975, *Nature* 256: 495-497). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for human HG51. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with human HG51, as described above. Human HG51-specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with an appropriate concentration of human HG51 protein or a synthetic peptide generated from a portion of human HG51 with or without an immune adjuvant.

Preimmune serum is collected prior to the first immunization. Each animal receives between about 0.1 mg and about 1000 mg of human HG51 protein associated with an acceptable immune adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of human HG51 protein or peptide fragment thereof in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. Those animals receiving booster injections are generally given an equal amount of human HG51 in Freund's incomplete adjuvant by the same route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20°C.

Monoclonal antibodies (mAb) reactive with human HG51 are prepared by immunizing inbred mice, preferably Balb/c, with human HG51 protein. The mice are immunized by the IP or SC route with about 1 mg to about 100 mg, preferably

about 10 mg, of human HG51 protein in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 1 to about 100 mg of human HG51 in a buffer solution such as phosphate buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol, about 1000 mol. wt., at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid phase immunoradioassay (SPIRA) using human HG51 as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, 1973, Soft Agar Techniques, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press.

Monoclonal antibodies are produced *in vivo* by injection of pristine primed Balb/c mice, approximately 0.5 ml per mouse, with about 2 x 10^6 to about 6 x 10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art.

In vitro production of anti-human HG51 mAb is carried out by growing the hybridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of human HG51 in body fluids or tissue and cell extracts.

It is readily apparent to those skilled in the art that the above described methods for producing monospecific antibodies may be utilized to produce antibodies specific for human HG51 peptide fragments, or full-length human HG51.

Human HG51 antibody affinity columns are made, for example, by adding the antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) and the cell culture supernatants or cell extracts containing full-length human HG51 or human HG51 protein fragments are slowly passed through the column. The column is then washed with phosphate buffered saline until the optical density (A_{280}) falls to background, then the protein is eluted with 0.23 M glycine-HCl (pH 2.6). The purified human HG51 protein is then dialyzed against phosphate buffered saline.

The specificity of binding of compounds showing affinity for HG51 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to HG51 or that inhibit the binding of a known, radiolabeled ligand of HG51 to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for HG51. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of HG51 and may be peptides, proteins, or non-proteinaceous organic molecules.

As a further modification of the above-described methods, RNA encoding HG51 can be prepared as, *e.g.*, by *in vitro* transcription using a plasmid

containing HG51 under the control of a bacteriophage T7 promoter, and the RNA can be microinjected into *Xenopus* oocytes in order to cause the expression of HG51 in the oocytes. Substances are then tested for binding to the HG51 expressed in the oocytes. Alternatively, rather than detecting binding, the effect of the substances on the electrophysiological properties of the oocytes can be determined.

The present invention includes assays by which HG51 agonists and antagonists may be identified by their ability to stimulate or antagonize a functional response mediated by HG51. HG51 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the Gα subunit of the G-protein to disassociate from the Gβ and Gγ subunits. The Gα subunit can then go on to activate a variety of second messenger systems.

Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR, it is necessary to ensure that the proper G-protein is present in the system containing the GPCR. It has been found, however, that there are certain G-proteins that are "promiscuous." These promiscuous G-proteins will couple to, and thus transduce a functional signal from, virtually any GPCR. See Offermanns & Simon, 1995, *J. Biol. Chem.* 270:15175-15180. These authors describe a system in which cells are transfected with expression vectors that result in the expression of one of a large number of GPCRs as well as the expression of one of the promiscuous G-proteins Gα15 or Gα16. Upon the addition of an agonist of the GPCR to the transfected cells, the GPCR was activated and was able, via Gα15 or Gα16, to activate the β isoform of phospholipase C, leading to an increase in inositol phosphate levels in the cells. Therefore, by making use of these promiscuous G-proteins as in Offermanns and Simon, *supra*, it is possible to set up functional assays for HG51, even in the absence of knowledge of the G-protein with which HG51 is coupled *in vivo*. One possibility is to create a fusion or chimeric protein composed of the extracellular and membrane spanning portion of HG51 fused to a promiscuous G-protein. Such a fusion protein would be expected to transduce a signal following binding of ligand to the HG51 portion of the fusion protein. Accordingly, the present invention provides a method of identifying antagonists of HG51 comprising:

(a) providing cells that expresses a chimeric HG51 protein fused at its C-terminus to a promiscuous G-protein;

(b) exposing the cells to an agonist of HG51;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG51; and,

(d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG51.

Another possibility for utilizing promiscuous G-proteins in connection with HG51 includes a method of identifying agonists of HG51 comprising:

(a) providing cells that expresses both HG51 and a promiscuous G-protein;

(b) exposing the cells to a substance that is a suspected agonist of HG51; and,

(c) measuring the level of inositol phosphates in the cells; where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of HG51.

Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG51 and the promiscuous G-protein in the cells.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16. Expression vectors containing Gα15 or Gα16 are known in the art. See, *e.g.*,

Offermanns; Buhl *et al.*, 1993, *FEBS Lett.* 323:132-134; Amatruda *et al.*, 1993, *J. Biol. Chem.* 268:10139-10144.

The above-described assay can be easily modified to form a method to identify antagonists of HG51. Such a method is also part of the present invention and comprises:

(a) providing cells that expresses both HG51 and a promiscuous G-protein;

(b) exposing the cells to a substance that is an agonist of HG51;

(c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG51; and,

(d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of HG51.

The conditions under which steps (b) and (c) of the method are practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG51 and the promiscuous G-protein in the cells.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16.

Agonists and antagonists of HG51 that are identified by the above-described methods should have utility in the treatment of diseases that involve the inappropriate expression of HG51. In particular, given the expression pattern of HG51 (see Figure 4), such agonists and antagonists should have utility in the treatment of various disorders including but not limited to obesity, type II diabetes, as well as various GI diseases such as inflammatory bowel disease, constipation and diarrhea.

The DNA of the present invention, or hybridization probes based upon the DNA, can be used in chromosomal mapping studies in order to identify the

chromosomal locations of the HG51 gene or of genes encoding proteins related to HG51. Such mapping studies can be carried out using well-known genetic and/or chromosomal mapping techniques such as, *e.g.*, linkage analysis with respect to known chromosomal markers or *in situ* hybridization. See, *e.g.*, Verma et al., 1988, Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY. After identifying the chromosomal location of the HG51 gene or genes encoding proteins related to HG51, this information can be compared with the locations of known disease-causing genes contained in genetic map data (such as the data found in the Genome Issue of Science, 1994, 265:1981-2144). In this way, one can correlate the chromosomal location of the HG51 gene or of genes encoding proteins related to HG51 with the locations of known disease-causing genes and thus help to limit the region of DNA containing such disease-causing genes. This will simplify the process of cloning such disease-causing genes. Also, once linkage between the chromosomal location of the HG51 gene or of genes encoding proteins related to HG51 and the locations of a known disease-causing gene is established, that linkage can be used diagnostically to identify restriction fragment length polymorphisms (RFLPs) in the vicinity of the HG51 gene or of genes encoding proteins related to HG51. Such RFLPs will be associated with the disease-causing gene and thus can be used to identify individuals carrying the disease-causing gene.

For such chromosomal mapping studies as described herein, it may be advantageous to use, in addition to the DNA of the present invention, the reverse complement of the DNA of the present invention or RNA corresponding to the DNA of the present invention.

Gene therapy may be used to introduce HG51 polypeptides into the cells of target organs. Nucleotides encoding HG51 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding HG51 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with HG51 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate HG51 activity.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM $MgCl_2$, 200 µM for each dNTP, 50 mM KCl, 0.2 µM for each primer, 10 ng of DNA template, 0.05 units/µl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press .

The assays described above can be carried out with cells that have been transiently or stably transfected or stably transformed with expression vectors which encode HG51. Transfection is meant to include any method known in the art for introducing HG51 into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing HG51, and electroporation. Transformation is meant to encompass a genetic change to the target cell resulting from an incorporation of DNA.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of human HG51. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of human HG51. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant HG51 or anti-HG51 antibodies suitable for detecting human HG51. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

Pharmaceutically useful compositions comprising modulators of human HG51 may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA,

modified human HG51, or either HG51 agonists or antagonists including tyrosine kinase activators or inhibitors.

Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular.

The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents may be desirable.

The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds for the

present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

EXAMPLE 1

Isolation and Characterization of DNA Fragments Encoding HG51

The full-length coding sequence of HG51 was isolated using a modified method called RCCA (Liu *et al.*, *Gene* 207 (1998) 1-7; McDonald et al., *BBRC* 247 (1998) 266-270). An EST was identified as a putative G protein coupled receptor and was designated HG51-EST.

HG51-EST: GenBank Acc. #: AA745052
EST Id: 1460712; EST name: np72h03.s1;
GenBank gi: 2783816; Clone Id: IMAGE :1131893 (3'):

TAAGTCAGTA GCATAAAAAC ATGAGCAAGT ACATCTAATC ACATCTGAGA ATACTAAAAT
GGATGTGTGG TTTCATTTCT GCATTTCATC TTAGCAGTAA ATGTCAAAAT GCATCATATA
TGCATTTGTG ACTGGAACTC TTCTCGAAGA GGCTGCCGCT AAACCCGTCC CACACGCAGC
CCACGGTGTC CCACACCCAG CCGTTCCTCA GGCAGGACAC GAAGGTAAAG GTGACCCCGA
AGAGGTACAC AGCAGGTCGC TGAGGCTGAT GTTGACCAGG AGGAGGTGAG TGGGAGTGCG
GAGCGCTGGA ACTTGTAGTA GAGGACGAGC ACCAGCAGGT TGTTGCCGAC GCCCAGCAGC
CCAATGGAGC CCAGCAGCAG CGCAGGCCCT CGTGC (SEQ ID NO:3).

The above EST may also be viewed at the National Center for Biotechnology Information (NCBI) homepage at http://www.ncbi.nlm. nih.gov/.

Four primers HG51.F39, HG51.F51. HG51.R121 and HG51.R179 were designed from the EST sequence. Primer pairs HG51.F39 + HG51.R121 and HG51.F51 + HG51.R179 were screened by PCR against cDNA libraries of placenta, prostate, testis, and fetal brain. The fetal brain and testis cDNA libraries was chosen for attempts to clone the full-length sequence of HG51. PCR reactions using Primers HG51.F51 and HG51.R179 were carried out on the fetal brain and testis cDNA libraries with ≥ 2.5 kb inserts. Positive wells were identified and RCCA was then performed on these wells with the following primer combinations HG51.F39 + PBS.543R or PBS.873F and HG51.R179 + PBS.543R or PBS.873F. PCR products were then purified, sequenced, and assembled to the contig. HG51.F51 + HG51.R179 primers were the used to screen the fetal brain 1-2.5kb and placental 1-2.5kb plasmid libraries. Positive wells were identified and RCCA was performed on these wells using the following primers combinations HG51.F51 + PBS.543R or PBS.873F and HG51.R179 + PBS.543R or PBS.873F in the primary reaction and in the secondary reaction nested primers HG51.F51 + PBS.578R or PBS.838F and HG51.R121 + PBS.578R or PBS.838F were used. Products were then purified, sequenced, and assembled to the contig. RCCA using the primer combinations above was then performed on new pools and products were purified and sequenced. An open reading frame of 1209 base pairs and encoding 402 amino acids was identified. Finally, the full length gene was amplified by semi-nested PCR reactions using primers HG51.KpnI + HG51.NotI1283R and HG51.KpnI + HG51.NotI1225R, and the product containing the sequence from HG51.KpnI to HG51.NotI1225 was cloned into pcDNA3.1.

The oligonucleotide primers utilized as described above are as follows:

HG51.KpnI: CGGGTACCATGTACTCGGGGAACCGCA (SEQ ID NO:4);

HG51.NotI1283R	GCGCGGCCGCACGGGTATTCCAGACACTTC (SEQ ID NO:5);
HG51.NotI1225R	GCGCGGCCGCCCCATCTTTCGTTGCCATTC (SEQ ID NO:6);
HG51.F51	CAACAACCTGCTGGTGCTCGTC (SEQ ID NO:7);
HG51.F39	GCTGGGCGTCGGCAACAA (SEQ ID NO:8);
HG51.R179	CAGGCAGGACACGAAGGTAA (SEQ ID NO:9);
HG51.R121	GGTCGCTGAGGCTGATGTTGAC (SEQ ID NO:10);
PBS.543R	GGGGATGTGCTGCAAGGCGA (SEQ ID NO:11);
PBS.578R	CCAGGGTTTTCCCAGTCACGAC (SEQ ID NO:12);
PBS.873F	CCCAGGCTTTACACTTTATGCTTCC (SEQ ID NO:13);
PBS.838F	TTGTGTGGAATTGTGAGCGGATAAC (SEQ ID NO:14).

The full length human HG51 cDNA disclosed herein as SEQ ID NO:1 is 1536 bp, with an open reading frame from nucleotide 160 to nucleotide 1365, with a "TAG" termination codon from nucleotides 1366-1368. This open reading frame encodes a human HG51 GPCR, which shares homology to human rhodopsin. The HG51 protein contains an open reading frame of 402 amino acids in length, as shown in Figure 2 and as set forth in SEQ ID NO:2. Figures 3A, 3B and 3C show the translation of the HG51 open reading frame. The nucleotide sequence shown is as set forth in SEQ ID NO:1. The amino acid sequence shown is as set forth in SEQ ID NO: 2.

EXAMPLE 2

Northern Analysis of Human HG51 Gene Expression

Human multi-tissue Northern blots were purchased from Clontech (Palo Alto, CA, USA). The entire coding region of HG51 was labeled with ^{32}P-dCTP using the Redy-Prime Kit of Amershem (Amersham, USA). Hybridizations and washing of filters were carried out under stringent conditions following the protocols of the Northern blot supplier (Clontech, Inc.). The blots were exposed to X-ray film by autoradiography for 5 days at -80°C with an intensifying screen. Northern analysis of multi-tissue mRNA blots showed that HG51 was weakly expressed in many tissues as transcripts of ~9.0 kb and ~2.5 kb. This data is presented in Figures 4A – 4E. Also, expression of HG51 in HEK293 cells led to the increase in intracellular cAMP level, indicating that HG51 is coupled to the Gs protein.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.